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In vitro induction and proliferation of adventitious roots in pineapple (*Ananas comosus* L.) cultivars of smooth cayenne and morris

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Abstract

The effect of shoot age (6 and 10-month-old) in quarter, half and full strength of two MS medium types (solid, liquid) supplemented with different concentrations of auxins and cytokinins on two pineapple cultivars (Smooth Cayenne and Morris) were examined to identify the optimal combination for root formation. All the shoots of both cultivars rooted in solid medium enriched with 2.0 mg/L IBA at quarter strength for Smooth Cayenne and half strength for Morris. Full strength solid medium enriched with 1.0 mg/L NAA was the best choice for rooting of both Smooth Cayenne and Morris, resulting in the tallest plantlets (74.1 mm), highest number of roots per shoot (11.4) and intermediate rooting percentage (89.5%). Neither shoot ages nor hormone types and concentrations had a significant effect on the rooting percentage. However, the largest number of roots was obtained from 6-month-old shoots treated with 1.0 mg/L NAA. Solid medium could be used to induce 100 % rooting and formation of 5 to 7 roots per shoot. In liquid medium, shoots failed to form roots in media containing NAA, while 100 % rooting with 4 to 5 roots per shoot were obtained in medium enriched with IAA and IBA.

Keywords: *Ananas comosus*, *in vitro*, shoots age, medium types, auxins, cultivars.
Abbreviations: BAP; 6-Benzylaminopurine, IBA; Indole-3-butyric acid,NAA; 1-Naphthaleneacetic acid, IAA; Indole-3-acetic acid, MS; Murashige and Skoog.

Introduction

Adventitious root formation is a complex process that is affected by multiple endogenous factors including phytohormones and environmental factors (Xuan et al., 2008). Morris pineapple is one of the most commonly grown local cultivar in Malaysia. There are only two micropro-pagation reports of this cultivar but the rooting process was not reported (Hamad and Taha, 2003; De Silva et al., 2006). For *in vitro* rooting of Smooth Cayenne pineapple, some researches have investigated different types of hormones and medium. MS phytagel solidified hormone free (Ko et al., 2006), enriched with NAA at 1.0 mg/L (Hamad and Taha, 2008) and combination of 2 mg/L IBA and 2 mg/L IAA (Bhatia and Ashwath, 2002) led to root induction. Half strength solidified MS media without hormone (Zepeda and Sagawa, 1981) and enriched with combination of 0.5 mg/L IBA and 0.5 mg/L NAA (Firoozabady and Gutterson, 2003) also showed suitable result of rooting process. Full strength solid media without hormone (Almieda et al., 1997), enriched with combination of 0.5 mg/L IBA and 0.5 mg/L NAA (Kanso et al., 2008) and half strength solid MS included2.0 mg/L IBA (Akbar et al., 2003) were suggested for rooting of Primavita, MD2 and Madhupur cultivars, respectively. For Elite cultivar, Gangopadahayay et al., (2005) used full strength liquid MS media enriched with 2.0 mg/L IBA 2.0 and 0.4 mg/L KN. Although, previous researchers used two (Fitchet, 1990; Kofi et al., 1993; Khatun et al., 1997; Rahman et al., 2001, Akbar et al., 2003), three (Dewald et al., 1988) and even four cultivars during testing of multiplication, no details were provided about the similarity or a particular pattern in rooting responses among the different cultivars.

Although published literatures concluded that shoots age could significantly affect the rooting responses during the subsequent stages (Kanso et al., 2008) and also the importance of shoot size for *ex vitro* survival has been confirmed (Escalona et al., 1999), however, the effects of the rooting treatments on plantlets height were rarely reported (Be and Debergh, 2006) and the shoot age effect was totally ignored. Hence, the main objectives of this study were 1. to investigate the effect of shoots age, auxin types and concentrations on all the three rooting parameters as well as the plantlets height of Smooth Cayenne and Morris pineapple 2. To search for treatment that could simultaneously induce rooting and plantlets elongation

Results

Effect of different auxin types and concentrations

Analysis of variance (Table 1) indicated that the cultivars had significant direct effect on root number, root length and plantlets height. Although interaction of hormone types and concentrations had no significant relation with root length, however, it had significant indirect effect on root number and plantlets height. Mean comparison (Table 2) showed that two cultivars reacted differently with hormone types and concentrations. Both cultivars could be rooted in several concentrations of 0.5 mg/L and 2.5 mg/L IBA and at 0.5, 1.0 and 2.5 mg/L NAA. Approximately, the rooting percentage was 71.4% in IBA and NAA for Smooth Cayenne. However, for Morris, rooting percentage in IBA was higher than NAA

Table 1. ANOVA of cultivars, auxin types and concentration for *in vitro* rooting of pineapple.

		p-values of MS			
S.O.V	df	Rooting %	Root No.	Root length	Plantlet height
Cultivars	1	0.62	0.001	0.003	0.001
Hormones types	1	0.07	0.001	0.056	0.963
Concentration	5	0.25	0.001	0.099	0.191
Cultivar*Hormones	1	0.06	0.001	0.395	0.001
Cultivar*Concentration	5	0.82	0.001	0.165	0.017
Hormones*Concentration	5	0.19	0.001	0.021	0.305
Cultivars*Hormones*Concentration	5	0.68	0.001	0.670	0.175

Non- significant ($p>0.05$); significant at 0.05 probability level ($0.01<p<0.05$); significant at 0.01 probability level ($p<0.01$)
Cultivar*Hormones; Interaction effect between cultivars and hormones.

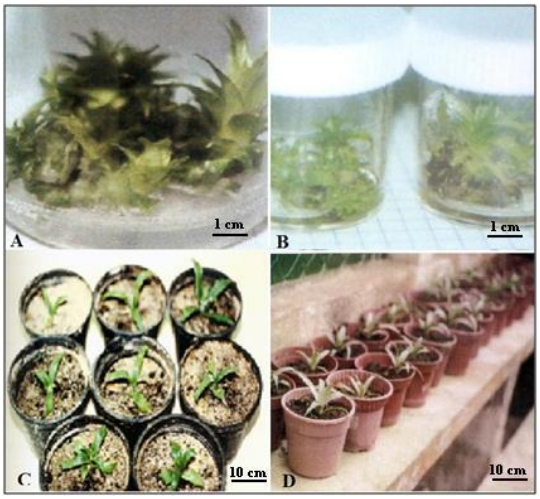

Fig 1. Pineapple regeneration process. A. Development of roots from the base of microshoots on solid MS medium, B. Development and elongation of shoots on liquid MS medium, C. Regenerated plantlets grown on soil after two weeks, D. Regenerated plantlets grown on soil in green house after 1 month.

with 81.6%. All the NAA concentrations (except 1.0 mg/L) produced roots shorter than hormone free (inhibited) in both Smooth Cayenne and Morris. Smooth Cayenne produced roots longer than Morris in NAA concentrations. At equal concentrations, except 1.0 mg/L, both of the cultivars (Smooth Cayenne and Morris) produced longer roots on IBA than NAA. Although presence of hormones (NAA, IAA and IBA) suppressed the root elongation of both cultivars, Smooth Cayenne had higher tendency to develop and progress better than Morris. Based on root length, 0.5 mg/L IBA would be recommended for Morris and 1.0 mg/L NAA for Smooth Cayenne. At equal concentration, results of IBA on Morris plantlets were taller than NAA. In Smooth Cayenne, both hormones were equally effective but the root number (14.5 roots) and plantlets height (46.1 mm) in NAA was significantly higher than (2.8 roots and 35.7 mm plantlets height) IBA. On the contrary, all aspect of rooting of IBA (81.6 %, 4.4 root each 7.7 mm long and 38.8 mm plantlets height) were higher than NAA (54 %, 2.4 roots each 4.4 mm long and 28 mm plantlets height) in Smooth Cayenne. In NAA medium, Smooth Cayenne had higher rooting percentage (71.4 %), more roots (14.5 roots), longer (9 mm) roots and taller (46.1 mm) plantlets than Morris (54 %, 2.4 roots, 4.4 mm, 28 mm, respectively). However, on IBA concentrations, Morris had higher rooting percentage (81.6 %) and more roots (4.4 roots) than Smooth Cayenne but there were no significant difference between them in root length and plantlet height. The best treatment for Smooth Cayenne was 1.0 mg/l NAA and IBA at 0.5 and 2.0 mg/L were the best treatment for Morris. These superior concentrations were used in the second experiment for assessment of medium strength on rooting responses of both cultivars.

Effect of different medium strength

Mean comparison (Table 3) showed that the combinations of medium strength, hormone types and concentrations could be grouped into five based on rooting percentage. About 85.8 % of the shoots in both cultivars formed roots in quarter strength-hormone free, quarter strength-enriched with 0.5 mg/L IBA and in full strength-enriched with 1.0 mg/L NAA. Smooth Cayenne cultivar on quarter strength enriched with 2.0 mg/L IBA and 1.0 mg/L NAA and full strength enriched with 0.5 mg/L IBA had higher rooting percentage than Morris, while Morris in half strength enriched with IBA at 0.5 and 2.0 mg/L had higher percentage than Smooth Cayenne. On hormone free medium, reducing the strength medium from full to half, increased the rooting percentage of Smooth Cayenne and decreased the rooting on medium enriched with 0.5 mg/L IBA. The highest number of root (12.8 and 10.0) from both cultivars obtained in MS medium enriched with 1.0 mg/L NAA. However, the strength medium could increase or decrease the root number of Morris cultivar depending on the hormone treatment. In both cultivars, quarter strength resulted in long roots and full strength gave short roots irrespective of hormone treatments. Smooth Cayenne produced the longest roots (27.7 mm) in quarter strength medium enriched with 1.0 mg/L NAA and Morris (64.7 mm) in quarter strength enriched with 0.5 mg/L IBA. In quarter and half strength media, Morris produced longer roots than Smooth Cayenne in medium enriched with IBA. It can be suggested that the root formation process of Smooth Cayenne is more responsive to hormone type than Morris while the root formation of Morris is more sensitive to change in medium strength than Smooth Cayenne. The tallest

Table 2. Mean comparison of cultivars, auxin types and concentration for *in vitro* rooting of pineapple.

	IBA			NAA		
(mg/L)	Smooth	Morris	Average	Smooth	Morris	Average
Rooting %						
0	61.3 ab	66.7 ab	64 ABC	61.3 ab	66.7 ab	64 ABC
0.5	83.3 a	100 a	91.7 AB	56.0 ab	45.0 ab	50.5 BC
1	66.7 ab	77.7 a	72.2 ABC	100 a	89.0 a	94.5 A
1.5	66.7 ab	67.0ab	66.8 ABC	67.0 ab	11.7 b	39.3 C
2	66.7 ab	100 a	83.3 AB	89.0 a	56.0 ab	72.5ABC
2.5	83.3 a	78.0 a	80.7 ABC	55.7 ab	55.3 ab	55.5 ABC
Root number						
0	2.0 a	3.3 a	2.7 A	2.0 c	3.3 a	2.7 B
0.5	2.7 a	6.3 a	4.5 A	3.0 c	1.4 a	2.2 B
1	2.0 a	3.0 a	2.5 A	29.7 a	4.3 a	17 A
1.5	3.0 a	3.7 a	3.4 A	17.3 b	0.4 a	8.9 AB
2	2.3 a	6.0 a	4.2 A	16.7 b	1.4 a	9.0 AB
2.5	5.0 a	4.0 a	4.5 A	18.3 b	3.7 a	11 AB
Root length (mm)						
0	10.3 abc	4.7 cd	7.5 ABC	10.3 abc	4.7 cd	7.5 ABC
0.5	9.0 a-d	15.3 ab	12.2 AB	5.4 cd	3.0 cd	4.2 C
1	12.7 abc	4.7 cd	8.7 ABC	17.7 a	10.3 abc	14 A
1.5	8.3 a-d	8.0 bcd	8.2 ABC	4.3 cd	0.4 d	2.4 C
2	9.3 a-d	9.3 a-d	9.3 ABC	8.0 bcd	4.0 cd	6.0 BC
2.5	12.0 abc	5.0 cd	8.5 ABC	6.3 bcd	4.0 cd	5.2 BC
Plantlet height (mm)						
0	34.3 cd	30 cd	27.2 A	34.3 cd	30 cd	27.2 A
0.5	41.7 bcd	40 bcd	40.9 A	35 cd	38.7 bcd	36.9 A
1	34.3 cd	36 cd	35.2 A	71.3 a	28.0 cd	49.7 A
1.5	24.7 cd	47.3 bc	36.0 A	30.3 cd	20.3 d	25.3 A
2	30.0 cd	44 bcd	37.0 A	43.3 bcd	30.3 cd	36.8 A
2.5	49.0 bc	35.3 cd	42.2 A	59.7 ab	22.0 d	40.9 A

The mean of the parameters with same small letters were not significantly different as per Duncan's multi-range test at $P<0.05$
The total mean of the concentration with same capital letters were not significantly different

plantlets of Smooth Cayenne (99.2 mm) was obtained in full strength medium enriched with 1.0 mg/L NAA, while the tallest of Morris (58.3 mm) was obtained in quarter strength enriched with 2.0 mg/L IBA. For both cultivars, reducing the medium strength enriched with NAA decreased the plantlets height, while it had no effect on medium enriched with 0.5 mg/L IBA.

Effect of shoot ages

Two ways analysis of variance (Table 4) indicated that the rooting percentage was not affected by hormones types, shoot age and their interactions. On the other hand, root length was only affected by shoot age and interaction of the shoot age and hormones. On the contrary, number of roots was affected by the hormone types and interaction of the different hormones and shoot age. Statistical analysis of each hormone as a separate experiment indicated that rooting was more sensitive to shoot age rather than hormone. Shoot age significantly affected all rooting aspects except the root length of the IBA treated samples and rooting percentage of the NAA treated samples (Fig. 1A). Although NAA had no effect on rooting percentage and root length, however, NAA induced a significant effect through interaction with shoot age on root number and plantlet height. IBA had no significant effect in rooting percentage, root number, root length and it only had indirect significant effect on plantlet height through interaction with shoot age. Mean comparison results of all combinations of shoot ages, hormones and concentrations by the Duncan test did not detect any significant differences in rooting percentage between the hormone concentrations (Table 5). None of the 6-month-old shoots treated with IBA produced 100 % rooting, while all of the 10-month-old shoots treated with IBA at 0.5, 1.0 and1.5 mg/L produced roots. In terms of rooting percentage, hormone free medium could be recommended as the best treatment for Smooth Cayenne. The root length of the 6-month-old shoots at all IBA levels, except 1.5 mg/L and the plantlets heights except at 0.5 mg/L were not significantly different from hormone free media. At all IBA concentrations, the 10-month-old shoots produced more roots and taller plantlets, except at 2.0 mg/L. The rooting responses to NAA were different from that of IBA. Compared to hormone free samples, NAA showed more roots per shoot in both ages, particularly in the 6-month-old shoots. Six-month-old shoots treated with 1.0 mg/L NAA produced 30 roots (the highest number of roots of all treatments).The roots per shoot decreased to 17 when the NAA concentrations were increased to 1.5 and 2.0 mg/L. The best treatment was 10-month old shoots treated with 1.5 mg/L NAA which produced the tallest plantlets (129 mm) and the longest roots (29.7 mm).The best hormone for rooting of Smooth Cayenne was NAA but the concentration should be adjusted according to shoot age. In this regard, 1.5 mg/L NAA for the 10-month-old and 1.0 mg/L NAA for the 6-month-old shoots were recommended.

Effect of different medium types

The data from each medium state were analysed separately and the analysis of variance (Table 6) showed significant difference between hormone types and concentrations. Mean comparison of the treatments in solid medium (Table 7, Fig. 1A) and liquid medium (Table8, Fig. 1B) showed significant difference by Duncan's Multiple Range test at $p\leq0.05$.

Table 3. Mean comparison of hormones types, concentrations and MS strength for *in vitro* rooting of pineapple.

Hormones	Concen.	MS	Pineapple cultivars					
	(mg/L)	(Strength)	Smooth	Morris	Average	Smooth	Morris	Average
			Rooting %			Root No.		
Free	0	Quarter	83.3 ab	89 ab	86.2 A	3 e	4 de	3.5 BC
		Half	83.3 ab	30 c	56.7 A	3 e	2 e	2.5 C
		Full	61.3 abc	78 ab	69.6 A	1.7 e	4 de	2.9 BC
IBA	0.5	Quarter	83.3 ab	89 ab	86.2 A	2 e	5 cde	3.5 BC
		Half	50.3 bc	59 abc	54.7 A	2 e	4 de	3.0 BC
		Full	83.3 ab	44.7 bc	64.0A	3 e	2 e	2.5 C
	2.5	Quarter	100 a	78 ab	89.0A	2 e	3 e	2.5 C
		Half	66.7 abc	100 a	83.3 A	3 e	5 cde	4.0 BC
		Full	72.3 abc	56 abc	64.2 A	3.7 de	3 e	3.3 BC
NAA	1	Quarter	91.7 ab	44.7 bc	68.2 A	7.3 bc	2 e	4.7 BC
		Half	91.7 ab	44.7 bc	68.2 A	6.7 cd	4 de	5.4 B
		Full	90.1 ab	89 ab	89.5 A	12.8 a	10 ab	11.4 A
			Root length (mm)			Plantlet height (mm)		
Free	0	Quarter	18 de	45.3 ab	31.7 A	31.7 ef	50.3 b-f	41 BC
		Half	20.7 de	15.3 e	18 ABC	39.7 c-f	29.3 f	34.5 C
		Full	11.1 e	13.7 e	12.4 BC	39.6 c-f	46.3 b-f	43 BC
IBA	0.5	Quarter	20 de	64.7 a	34.8 A	40.3 c-f	51 b-f	45.7 BC
		Half	14 e	24 de	19 ABC	36.7 def	47.3 b-f	42.2 BC
		Full	9 e	10.7 e	9.8 C	41.7 c-f	47.3 b-f	44.5 BC
	2.5	Quarter	11.7 e	43 abc	27.3 AB	38.3 def	58.3 bcd	48.3 BC
		Half	19 de	36.3 a-d	27.7 AB	38.3 def	56.7 b-e	47.5 BC
		Full	8.3 e	10.3 e	9.3 C	43.8 c-f	45.0 c-f	44.4 BC
NAA	1	Quarter	27.7 b-e	17.3 de	22.5 ABC	71.3 b	35.7 def	53.5 BC
		Half	23 de	14.7 e	18.8 ABC	65.7 bc	47.7 b-f	56.7 AB
		Full	25.6 cde	15.3 e	20.5 ABC	99.2 a	49.0 b-f	74.1 A

The mean of the parameters with same small letters were not significantly different as per Duncan's multi-range test at $P<0.05$
The total mean of the concentration with same capital letters were not significantly different.

Solid cultures

In solid cultures (Table 7), about 92.3 % of the shoots rooted in medium enriched with some concentrations of NAA (1.0, 3.0 and 4.0 mg/L), IAA (1.5, 2.5 and 5.0 mg/L) and IBA (0.5, 1.0, 2.0, 2.5, 3.5, 4.0 and 4.5 mg/L), while only 66.7 % of the shoots produced roots in hormone free media. The highest root formation was 7 roots per shoot obtained in medium enriched with 4.0 and 4.5 mg/LIBA. The first (17.7 mm) and second (15.3 mm) longest roots obtained in medium enriched with IBA at 4.0 and 0.5 mg/L, respectively. Since 4.5 mg/L IBA is too high, 0.5 mg/L IBA would be recommended for induction and development of roots in solid medium. The tallest plantlets (37.3 mm) obtained from shoots treated with 1.5 mg/L IBA and the shortest plantlets (11 mm) obtained on medium enriched with NAA at 1.5, 2.5 and 4.5 mg/L.

Liquid cultures

Using liquid cultures (Table 8), all of the shoots rooted in medium enriched with IAA (1.0, 1.5, 2.0, 2.5, 4.0 and 4.5 mg/L) and IBA (1.0, 1.5, 2.0, 3.0 and 3.5 mg/L). In liquid medium, 100 % rooting of Morris pineapple could be obtained in 11 different treatments and only IAA and IBA could be used for root induction. The highest root number with 5 roots per shoot obtained in 1.5 mg/L IAA and 3.5 mg/L IBA and the second roots number (4roots) obtained in medium enriched with IBA at 2.5, 3.0 mg/L and IAA at 1.0, 2.0, 5.0 mg/L. The longest roots (56 mm) observed in medium treated with 1.0 mg/L IBA. No root formed in all NAA concentrations except at 5.0 mg/L where the mean of roots length was 18 mm. NAA in liquid medium is definitely not suitable for rooting of Morris pineapple. Although the concentration is a little high, 3.5 mg/L IBA appeared to be the best compromise in liquid medium that maintained three rooting parameters at the highest values (Fig. 1B). The tallest plantlets (54.7 mm) were obtained in medium supplemented with 2.5 mg/L IAA. For the highest root formation per shoot and as the best compromise for rooting percentage, the medium should be solidified. Longer roots and taller plantlets were obtained in liquid medium, while more roots obtained in solid and there was no significant difference between them for rooting percentage. Except for root number, liquid medium was clearly superior to solid state medium. However, hormone type and concentration should be selected according to the rooting aspects which are research goals of a particular plant regeneration study (Fig. 1C, D).

Discussion

In vitro plant regeneration of pineapple cv. Smooth Cayenne had been reported by Hamad and Taha (2008); however, no detail on *in vitro* rooting of this cultivar was reported. Generally, the different cultivars and medium strength play major role in rooting of pineapple and the cultivars might be the most important factor that determined the rooting process

Table 4. ANOVA of main (direct) and interaction (indirect) effect of shoot ages and singly applied IBA and NAA for *in vitro* rooting of Smooth cayenne pineapple.

		p-values of rooting MS			
S.O.V	df	Rooting %	Root No.	Root length	Plant height
A). Together					
Shoot ages	1	0.061	0.324	0.001	0.001
Hormone types	1	0.631	0.001	0.104	0.001
Shoot ages*Hormones	1	0.187	0.009	0.049	0.088
Error	56				
Total	60				
B). Each hormone alone					
IBA					
Shoot ages	1	0.019	0.005	0.068	0.001
IBA conc.	4	0.591	0.271	0.275	0.104
Shoot ages*IBA conc.	4	0.851	0.584	0.218	0.049
Error	20				
Total	30				
NAA					
Shoot ages	1	0.737	0.001	0.001	0.001
NAA conc.	4	0.537	0.001	0.349	0.016
Shoot age*NAA conc.	4	0.583	0.001	0.128	0.017
Error	20				
Total	30				

Non- significant ($p>0.05$); significant at 0.05 probability level ($0.01<p<0.05$); significant at 0.01 probability level ($p<0.01$)
Shoot ages*Hormones; interaction effect between Shoot ages and hormones.

Table 5. Mean comparison of shoot ages and concentration of singly applied IBA and NAA for *in vitro* rooting of Smooth cayenne pineapple.

	IBA			NAA		
	6 months	10 months	Average	6 months	10 months	Average
Rooting %						
0	66.7 a	75.3 a	71.0 A	66.7 a	75.3 a	71.0A
0.5	83.3 a	100 a	91.7 A	56.0a	67.0 a	61.3 A
1	66.7 a	100 a	83.3 A	100 a	77.7 a	88.8 A
1.5	66.7 a	100 a	83.3 A	67.0a	100 a	83.5 A
2	66.7 a	83.3 a	75.0A	89.0a	78.0a	83.5 A
Root length (mm)						
0	12.3 bc	9.7 bc	11.0A	12.3 bc	9.7 bc	11.0 A
0.5	9 bc	14.7 abc	11.8 A	5.4 c	19 abc	12.2 A
1	12.7 bc	19.0abc	15.8 A	17.7 abc	24.3 ab	21.0A
1.5	8.3 c	20.0abc	14.2 A	4.3 c	29.3 a	16.8 A
2	9.3 bc	8.3 c	8.8 A	8 c	29.7 a	18.8 A
Root No						
0	2 d	2.3 d	2.2 A	2 d	2.3 d	1.1 B
0.5	3 d	5.7 cd	4.3 A	3 d	7.4 cd	5.2 B
1	2 d	6.3 cd	4.2 A	30 a	8.3 cd	19.2 A
1.5	3 d	7.7 cd	5.3 A	17 b	10.7 bc	13.8 A
2	2 d	4.3 cd	3.2 A	17 b	9.3 cd	13.2 A
Plantlet height (mm)						
0	34.3 gh	48.3 e-h	41.3 B	34.3 gh	48.3 e-h	41.3 B
0.5	41.7 fgh	71.3 c-f	56.5 A	35.0gh	108.3 ab	71.7 A
1	34.3 gh	69.7 def	52.0 AB	71.3 c-f	100.7 abc	86.0 A
1.5	24.7 h	76.7 cde	50.7 AB	30.3 gh	129.0 a	79.7 A
2	30.0gh	60.0 d-g	45.0 AB	43.3 fgh	87.3 bcd	65.3 AB

The mean of the parameters with same small letters were not significantly different as per Duncan's multi-range test at $P<0.05$
The total mean of the concentration with same capital letters were not significantly different

of initiation, development and plantlet growth. Statistically, in full strength medium (first experiment), all rooting parameters, except rooting percentage were mainly affected by cultivars. The two cultivars, Smooth Cayenne and Morris showed different and distinctive responses to hormone types. The highest rooting percentage, longest roots and tallest plantlets of Morris were obtained in IBA medium, while in both hormones (IBA and NAA) the root number was not significantly different from hormone free medium. Bhatia et al., (2002) did not try hormone free medium for rooting of Smooth cayenne and Akbar et al., (2003) reported that Madhupur pineapple could not root without hormone. NAA was recommended for Smooth Cayenne rooting (Hamad and Taha, 2008; Fitchet, 1990). Although our results also showed that the best rooting of Smooth Cayenne was in NAA medium, however NAA was not suitable for Morris, whereby it was even less effective than hormone free medium. In the second experiment, using MS medium at half and full strength produced roots in pineapple, while previous reports (Soneji et al., 2002) did not provide any details. Our results

Table 6. Significant of main and interaction effect of hormones and concentration on rooting parameters of Morris pineapple in solid and liquid MS medium.

Factors	df	p-values of rooting MS			
		RP	RN	RL	PH
Solid medium					
Hormones	2	0.068	0.001	0.001	0.001
Concentration	10	0.377	0.0921	0.569	0.001
Horm.*Concen.	20	0.116	0.0017	0.012	0.001
Error	66				
Total	99				
Liquid medium					
Hormones	2	0.001	0.001	0.001	0.001
Concentration	10	0.060	0.0799	0.013	0.106
Horm.*Concen.	20	0.012	0.0385	0.001	0.001
Error	66				
Total	99				

RP: Rooting percentage, RN: Root number, RL: Root length, PH: Plantlet height, Non- significant ($p>0.05$); significant at 0.05 probability level ($0.01<p<0.05$); significant at 0.01 probability level ($p<0.01$). Shoot ages*Hormones; Interaction effect between Shoot ages and hormones.

Table 7. Effect of different hormone types and concentration on rooting of Morris pineapple in solid medium.

Conc.	Hormones types							
(mg/L)	NAA	IAA	IBA	Average	NAA	IAA	IBA	Average
	Plantlets height (mm)				Rooting %			
0	19.3 jk	19.3 jk	19.3 jk	19.3 C	66.7 ab	66.7 ab	66.7 ab	7.0 AB
0.5	38.7 a-f	17.0 k	40 a-e	31.9 AB	44.7 abc	0 c	100 a	5.6 B
1	28 f-k	29.3 e-j	36 a-g	31.1 AB	89 a	66.7 ab	77.7 a	8.7 AB
1.5	20.3 ijk	28.3 e-k	47.3 a	32.0 AB	11 bc	100 a	66.7 ab	6.6 AB
2	30.3 e-ij	32 d-i	44 abc	35.4A	55.7 ab	66.7 ab	100 a	7.8 AB
2.5	21 ijk	29 e-j	35.3 b-h	28.4 B	59 ab	89.0 a	78.0 a	8.6 AB
3	30 e-j	31.3 e-i	37.3 a-f	32.9 AB	100 a	66.7 ab	55.3 ab	8.4 AB
3.5	31.3 e-i	29.7 e-j	43 a-d	34.7 AB	66.7 ab	59 ab	89.0 a	8.0 AB
4	37 a-g	25.3 g-k	45.3 ab	35.9 A	89.0 a	55.7 ab	100 a	9.0 A
4.5	22 h-k	31.3 e-i	38 a-f	30.4 AB	55.3 ab	66.7 ab	100 a	8.4 AB
5	34 b-h	28.3 e-k	33 c-i	31.8 AB	44.3 abc	89.0 a	66.7 ab	7.7 AB
	Roots No				Root length (mm)			
0	2 def	2 def	2 def	1.6 B	6 b-e	de	6 b-e	2.4 A
0.5	1 def	0 f	6 abc	1.7 AB	3 de	0 e	15.3 ab	2.3 A
1	4 a-e	3 b-f	3 b-f	2.1 AB	10 a-d	8.7 a-d	4.7 b-e	2.8 A
1.5	0 f	5 a-d	4 a-f	1.9 AB	0.3 e	10.7 a-d	8 a-e	2.5 A
2	1 def	3 b-f	6 abc	2.0 AB	4 cde	6.7 b-e	9.3 a-d	2.6 A
2.5	4 a-e	2 c-f	4 a-e	2.0 AB	4 cde	6.7 a-e	5 b-e	2.5 A
3	6 abc	3 b-f	3 b-f	2.2 A	9 a-d	6.3 a-e	6.3 b-e	2.7 A
3.5	4 a-f	1 def	5 abc	2.02 AB	5.3 b-e	4.3 cde	7 a-e	2.5 A
4	4 a-e	1 def	7 ab	2.1 A	8 a-d	4 cde	13.3 abc	3.0 A
4.5	1 def	3 b-f	7 ab	2.1 A	6.3 b-e	7.7 a-e	17.7 a	3.2 A
5	1 def	1 def	4 a-e	1.7 AB	3.3 de	6.3 a-e	7.7 a-e	2.5 A

The mean of the parameters with same small letters were not significantly different as per Duncan's multi-range test at $P<0.05$.
The total mean of the concentration with same capital letters were not significantly different.

supported using1.0 mg/l NAA in full strength medium for Smooth Cayenne as recommended by Hamad and Taha (2008) and Fitchet (1990) and 2.0 mg/L IBA in quarter strength medium for Morris. The responses to different medium strength indicated that some ions become toxic when their values are more than a certain level. It is clear that rooting treatment for one cultivar could not be generalized for other cultivars. Besides optimization of rooting for the two cultivars (Smooth Cayenne and Morris) and minimizing the cost, findings of this study may help in proper design of experiment for investigation of the different rooting responses. For both 6 and 10-month-old shoots, NAA was better than IBA, but the optimal concentration for older shoots was higher than the optimal concentration for younger shoots. The results supported 1.0 mg/L NAA as suggested by Hamad and Taha (2008) and Fitchet (1993) when the shoot age was 6 months old. In contrast, Be and Debergh (2006) reported 91.0 mm tall plantlets after 60 days of incubation in hormone free MS medium. Bhatia and Ashwath (2002) observed that rooting could occur in the multiplication medium if the stages continued for more than 6 subcultures. These reports confirmed our results that shoot age is an important factor for *in vitro* rooting of pineapples. The effect of IBA and NAA on rooting of Smooth Cayenne pineapple seemed to be through different dictated mechanisms by the shoot ages. Al-Saif et al. (2011) reported that the optimal concentration of BAP for multiplication of pineapple (*A. comosus* L. Merr.) cv. Smooth Cayenne appeared in solid MS. In a solid MS medium, 0.5 mg/L IBA was recommended for unnamed cultivar (Dolgov et al., 1998) and for Mauritius (Fernando, 1986). IBA at 1.0 mg/L was recommended for unnamed cultivar (Khan et al., 2004) and at 2.0 mg/L for Madhupur cultivar (Akbar et al., 2003) and Queen Pineapple (Devi et al., 1997). Our results demonstrated that at 0.5 mg/L IBA in solid medium was the best for all aspects of rooting of Morris pineapple, except for the plantlet height. This study

Table 8. Effect of different hormone types and concentration on rooting of Morris pineapple in liquid medium.

Conc.	Hormones types							
(mg/L)	NAA	IAA	IBA	Average	NAA	IAA	IBA	Average
	Plantlets height (mm)				Rooting %			
0	30.0 e-i	30 e-i	30 e-i	30.0 C	66.7 ab	66.7 ab	66.7 ab	66.7 A
0.5	22.3 hi	47 a-f	40 c-h	36.4 ABC	0 c	66.7 ab	33.3 bc	33.3 B
1	25 ghi	40.7 c-h	52.7 a-d	39.4 ABC	0 c	100 a	100 a	66.7 AB
1.5	21 i	46 b-f	51.7 a-d	39.6 ABC	0 c	100 a	100 a	66.7 AB
2	19.3 i	41.3 c-g	45 b-f	35.2 ABC	0 c	100 a	100 a	66.7 AB
2.5	23.3 ghi	64.7 a	36.3 d-i	41.4 AB	33.3 bc	100 a	66.7 ab	66.7 AB
3	28.3 f-i	55.3 abc	52.7 a-d	45.4 A	0 c	66.7 ab	100 a	55.6 AB
3.5	20 i	40.3 c-h	58 abc	38.8 ABC	0 c	55.3 ab	100 a	51.7 AB
4	20.7 i	47.7 a-e	35.7 d-i	34.7 BC	33.3 bc	66.7 ab	66.7 ab	55.6 AB
4.5	20.7 i	58 abc	29.3 e-i	36.0 ABC	0 c	100 a	0 c	33.3 B
5	23 ghi	62 ab	36 d-i	40.3 ABC	66.7 ab	100 a	66.7 ab	77.8 A
	Roots No.				Root length (mm)			
0	3 a-d	3 a-d	3 a-d	3.0 A	4.8 d-g	4.7 d-g	4.7 d-g	4.7 C
0.5	0 f	3 a-d	1 c-f	1.3 B	0 g	20.3 b-f	6.3 efg	8.8 C
1	0 f	4 ab	3 a-e	2.3 AB	0 g	30 abc	56 a	28.6 A
1.5	0 f	5a	2 a-f	2.3AB	0 g	16 b-f	17.3 b-f	11.1ABC
2	0 f	4 ab	2 a-f	2.0 AB	0 g	19 b-f	36.7 ab	18.5ABC
2.5	0 f	3 a-e	4 abc	2.3 AB	0 g	32.7 ab	34.3 a-e	22.3 AB
3	0 f	1 b-f	4 abc	1.6 B	0 g	6.0 d-g	26 a-e	10.6 BC
3.5	0 f	3 a-e	5a	2.6 AB	0 g	19.7 b-f	41.7 ab	20.5ABC
4	0 f	2 a-f	2 a-f	1.3 B	4 fg	21.7 b-f	18.7 b-g	14.8ABC
4.5	0 f	3 a-e	0 f	1.0 B	0 g	35.0 ab	0 g	11.6 BC
5	1 def	4 abc	1 b-f	2.0 AB	18.3 b-g	28.3 a-d	7.7 c-g	18.1ABC

The mean of the parameters with same small letters were not significantly different as per Duncan's multi-range test at P<0.05, The total mean of the concentration with same capital letters were not significantly different.

also demonstrated that effect of IAA in liquid MS medium was better than hormone free, IBA and NAA supplemented media. In most cases, parameters such as plantlet height are usually ignored as a factor of rooting assessment.

Materials and methods

Effect of different auxin types and concentrations

To prepare the Murashige and Skoog (1962) medium (MS), 3% (w/v) sucrose and 0.8% (w/v) agar were added to stock solution at different concentrations of IBA at 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l. The pH of the medium was adjusted to 5.6-5.8 using 1 N NaOH or 1N HCl. The jars covered with autoclavable lid and the medium autoclaved at 121 ℃ and pressure of 1.5 kg/cm^{2}for 25 minutes. Stock cultures of Smooth Cayenne and Morris that were grown in agar solidified MS medium enriched with 2.0 mg/L BAP were used as source of explants. The cultures were incubated under 16 and 8 hours photoperiod and constant temperature of 25±1°C after 60 days, cultures were taken out from the incubation room for counting of roots, measuring of root length and plantlets height. For each rooting parameter (Root percentage, Root number, Root length and Plantlet height), the results of each three culture tubes of the same treatment were averaged and used as one of three replicate per treatment. The rooting percentage computed and transformed to square root of x+1 before analysis. Analysis of variance and Duncan Multiple Range Test for treatments mean significance were done at probability level of 5 % using SPSS statistical.

Effect of different medium strength

According to obtained results of the rooting parameters from experiment 1, (0.5 mg/L and 2.0 mg/L) IBA and also 1.0 mg/L NAA were chosen for investigation of the medium strength effect. One and half litres of full strength MS medium were prepared and divided into 720, 360 and 180 ml in 3 beakers, respectively. The volume completed to 720 ml using distilled water to obtain medium of half and quarter strength and 30 g/L sucrose as well as 8 g/L agar added to each beaker. After autoclaved, the content of each jar was divided in the laminar flow chamber into 18 culture tubes. One shoot from Morris and Smooth Cayenne stock cultures was cultured in each tube. The cultures incubated under the same condition as experiment I and the data were recorded and analysed in the same manner.

Effect of shoot ages

Smooth Cayenne shoots from 6-month and 10-month-old stock cultures were used for *in vitro* rooting. These stocks were maintained by subculturing every 60 days in full strength agar solidified MS medium supplemented with2.23 mg/L BAP (9.9 µM). Shoots were separated and cultured individually in full strength MS medium enriched with30 g/L sucrose and either IBA or NAA at 0.0, 0.5, 1.0, 1.5 and 2.0 mg/L were added separately. The medium was adjusted to pH 5.7 and solidified with 7.0 g/L agar. There were 9 shoots per treatment and a total of 17 treatments were undertaken. After 60 days of incubation, root number, root length and plantlet height were measured and used for computing the rooting percentage of each treatment. Analysis of variance and mean separation were done using SPSS statistical and Duncan Multiple Range Test at probability level of 0.05.

Effect of different medium types

MS medium was prepared and NAA, IAA and IBA at (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mg/L) were added to the culture containers. Three shoots were cultured in each culture tube under laminar flow cabinet and transferred to culture room where they were maintained under photoperiod

of 16 hours of light and constant temperature of 25±1 °C. After two months of incubation, the cultures were taken out of the incubation room and the root number and length and plantlet height were recorded. The experiment was repeated using liquid medium. The data of the liquid medium transformed using square root of x+1. The results of the solid and liquid medium were analysed separately. The differences among treatments were investigated using four different indices (rooting percentage, root number, root length and plantlet height) and assessed by analysis of variance and Duncan multiple range test at 0.05 probabilities using SPSS statistical package.

Conclusion

It is clear that conclusion regarding the optimal hormone type and concentration for root induction could not be done based only on one rooting parameter and one medium state. Our recommendation would be much better if other factors such as sucrose content are taken into consideration. The present study showed that NAA is definitely not an option in liquid medium and only 3 of the 10 tested NAA levels could be used in solid medium. IAA was the best hormone in liquid medium for all aspects of rooting of Morris pineapple which was never recommended and never tried singly. Determining of concentrations and hormone types could be switched from promotion to inhibitory for further successful and informative study of rooting physiology in pineapple.

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